

HEMICELLULOSIC POLYSACCHARIDES FROM BAMBOO
SHOOT CELL-WALLS

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Abstract—Cell-wall polysaccharides of bamboo shoots were extracted with chelating reagent (CDTA, *trans*-1,2-cyclohexanediamine-*N,N,N',N'*-tetraacetic acid), Na₂CO₃ and KOH solutions. Glycosyl composition and glycosyl linkage analyses showed that the hemicellulose was composed of arabinoxylan, (1 → 3, 1 → 4)-β-D-glucan, xyloglucan and glucomannan. (1 → 3, 1 → 4)-β-D-glucan was purified by anion-exchange chromatography and xylanase-treatment. It consisted of 20–30% of 3-linked and 70–80% of 4-linked glucosyl residues, having a *M_r* range of 100 000–380 000. Hydrolysis of the glucan with a purified cellulase {*endo*-β-(1 → 4)-glucanase}, released glucose, cellobiose and Glcp-(1 → 4)-β-D-Glcp-(1 → 3)-β-D-Glcp. Oligosaccharides having contiguous 3-linked glucosyl residues were not detected. The structure of (1 → 3, 1 → 4)-β-D-glucan of bamboo shoot cell-walls was thus determined to be →3)-β-D-Glcp-[(1 → 4)-β-D-Glcp-]₂₋₄-(1 → . Xyloglucan was purified by anion-exchange chromatography, precipitation with iodide and gel-permeation chromatography. Arabinoxylan could not be separated completely by the procedure. The remaining arabinoxylan could not be degraded completely by xylanase treatment. Any linkage between arabinoxylan and xyloglucan would retard removal of arabinoxylan. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The properties of primary walls alter on account of synthesis and degradation of the cell-wall polysaccharides during plant cell growth [1]. Such turnover of the cell-wall polysaccharide is thought to regulate plant cell elongation. Furthermore, the bioactive oligosaccharides for regulation of plant cell growth and induction of phytoalexins were reportedly produced from primary cell-wall polysaccharides [1]. Therefore, structural characterization of primary cell-wall polysaccharides is important in the elucidation of mechanisms of plant cell growth and in the detection of bioactive oligosaccharides. Albersheim and co-workers have studied the cell-wall polysaccharides from dicot cells, such as suspension-cultured sycamore (*Acer pseudoplatanus*) cells, and have characterized pectic polysaccharides, arabinogalactan and xyloglucan [2]. Their structural charac-

terization of the cell-wall polysaccharides made it possible to isolate and identify bioactive oligosaccharides [3]. For example, xyloglucan oligosaccharides reportedly inhibited cell elongation induced by auxin [4]. Compared with the study of dicot cell-walls, the structural studies of cell-wall polysaccharides of monocots are limited [5]. Monocot cell-walls contain pectic polysaccharides, arabinoxylan, (1 → 3, 1 → 4)-β-D-glucan and small amounts of xyloglucan (*ca* 2% of cell-wall material) [5–10]. The (1 → 3, 1 → 4)-β-D-glucan and xyloglucan was determined by methylation analysis. Nevins and co-workers reported that the (1 → 3, 1 → 4)-β-D-glucan decreased during elongation of *Avena* coleoptiles and suggested that (1 → 3, 1 → 4)-β-D-glucan played an important role in monocot cell growth, in place of xyloglucan [11, 12]. Kato *et al.* isolated (1 → 3, 1 → 4)-β-D-glucan oligosaccharides from suspension-cultured rice cells [6] and showed the presence of β-D-glucan built up predominantly of 3-*O*-β-cellobiosyl-D-glucose and 3-*O*-β-cellotriosyl-D-glucose in bamboo shoot cell-walls by glycosyl linkage analysis and en-

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Table 1. Glycosyl compositions of fractions extracted from bamboo shoot cell-walls prepared in 1993 using chelating agent and alkaline solutions

Fraction	Yield*	Glycosyl composition (mol %)							
		Rha	Ara	Xyl	Man	Gal	Glc	Gal A	Glc A
Bamboo Shoot	—	0.7	14.8	19.6	1.1	5.5	58.1	trace	0
CDTA-I	10.1	1.7	11.0	5.4	0	14.1	59.5	8.5	0
CDTA-II	1.5	0	10.9	15.9	0	20.8	47.1	4.9	0
Na ₂ CO ₃ -I	3.6	1.1	19.1	23.8	0.8	22.3	23.4	8.1	1.3
Na ₂ CO ₃ -II	1.1	0.8	15.0	27.6	0	6.2	48.6	1.7	0
1 M KOH-I	7.3	0	6.8	16.0	0	1.6	75.4	0.2	0
1 M KOH-II	15.5	1.4	27.5	31.3	0.5	10.4	24.9	4.1	0
4 M KOH-II	7.2	0.5	13.8	22.0	0.4	7.4	54.7	1.2	0
4 M KOH-II	3.0	0.6	15.2	21.9	0.3	7.6	52.8	1.6	0
Residue	30.9	0.5	9.5	10.6	1.4	4.1	73.7	trace	0

*Wt% on cell-walls of bamboo shoots.

zymatic degradation studies [9]. Furthermore, they speculated from the analyses of xyloglucan oligosaccharides generated by enzymatic degradation of rice cell-walls that the structure of monocot xyloglucan was much simpler than that from dicots.

The present work describes the isolation and structural characterization of (1 → 3, 1 → 4)-β-D-glucan and xyloglucan from bamboo shoot cell-walls.

RESULTS AND DISCUSSION

The cell-walls of bamboo shoots consisted mainly of arabinosyl, xylosyl and glucosyl residues (Table 1). The cell-walls were treated with 0.05 M *trans*-1,2-cyclohexanediamine-*N,N,N',N'*-tetraacetic

acid (CDTA), 0.05 M Na₂CO₃, 1 M and 4 M KOH solutions to extract the cell-wall polysaccharides. Arabinoxylan precipitated during dialysis against de-ionized water. Therefore, the total yield of extracted polysaccharides and residue was 80.2%. Most of the pectic polysaccharides are extracted from dicot cell-walls with CDTA and Na₂CO₃ solutions [13]. The galacturonic acid contents of these extracts were 4.9–8.5%, showing that the amount of pectic polysaccharides in bamboo shoot cell-walls is lower than that in dicot cell-walls. Each extract contained terminal arabinosyl, 4-linked xylosyl, 3-linked and 4-linked glucosyl residues (Tables 2 and 3). The glycosyl linkages showed the presence of arabinoxylan and (1 → 3, 1 → 4)-β-D-glucan. Terminal xylosyl and 4,6-linked glucosyl residues

Table 2. Glycosyl linkage compositions of fractions extracted from bamboo shoot cell-walls prepared in 1993 using chelating reagent and alkaline solutions

Glycosyl Residue	Linkage	Fraction (mol %)							
		CDTA-I	CDTA-II	Na ₂ CO ₃ -I	Na ₂ CO ₃ -II	1 M KOH-I	1 M KOH-II	4 M KOH-I	4 M KOH-II
Rhamnosyl	2-linked	—	—	—	—	—	1.0	—	1.7
Arabinosyl	terminal-	—	7.6	23.3	17.9	3.9	6.8	11.1	9.7
	2-linked	—	—	3.2	1.6	—	—	1.1	—
Xylosyl	5-	1.7	3.9	7.7	13.0	1.6	5.4	8.2	9.2
	3,5-	—	—	—	—	—	—	1.9	1.5
	terminal-	—	—	—	—	1.4	1.7	2.6	4.2
Mannosyl	4-linked	7.3	2.7	0.9	6.2	3.9	4.1	5.5	5.2
	2,4-	3.7	—	—	—	—	—	—	—
	3,4-	11.1	14.1	4.5	18.9	5.9	6.1	13.1	13.0
Galactosyl	terminal-	—	trace	—	—	—	—	1.0	—
	4-linked	—	trace	—	1.8	—	0.2	7.1	3.9
	terminal	—	13.6	1.7	0.8	0.6	2.0	3.5	2.5
Glucosyl	4-linked	—	—	13.3	9.9	2.0	3.8	6.8	4.5
	6-	—	8.3	5.7	5.1	1.6	1.7	4.9	—
	2,3-	—	—	1.9	—	—	—	—	—
	3,4-	—	—	5.6	—	—	—	—	—
	3,6-	16.8	—	10.5	4.9	trace	—	—	—
	terminal-	—	12.1	1.8	0.7	0.5	—	0.6	1.5
Glucosyl	3-linked	13.9	15.8	3.0	3.8	19.1	9.6	9.2	5.8
	4-	45.4	21.7	14.1	15.5	52.2	33.6	6.8	27.3
	2,4-	—	—	trace	—	0.9	5.0	6.8	trace
	2,6-	—	—	—	—	2.1	—	trace	—
	3,4-	—	—	2.6	—	trace	6.5	2.5	10.1
	3,6-	—	—	—	—	1.0	1.9	—	—
	4,6-	—	trace	—	—	—	7.0	12.2	—
	3,4,6-	—	—	—	—	3.3	—	—	—

Table 3. Glycosyl linkage compositions of fractions extracted from bamboo shoot cell-walls prepared in 1994 using alkaline solutions

Glycosyl Residue	Linkage	Fraction (mol %)*					
		Na ₂ CO ₃ -I	Na ₂ CO ₃ -II	1 M KOH-I	1 M KOH-II	4 M KOH-I	4 M KOH-II
Rhamnosyl	2-Linked	2.0	2.2	2.3	1.6	1.2	1.9
	2,3-	0.1	—	—	—	—	—
	2,4-	1.7	1.7	1.8	2.0	2.5	2.6
Fucosyl	Terminal	—	—	—	0.3	—	—
Arabinosyl	Terminal	24.1	26.3	12.3	7.1	5.2	4.1
	3-Linked	1.0	1.0	0.5	0.3	0.2	4.1
	5-	2.2	2.5	4.5	1.0	0.3	4.1
Xylosyl	3,5-	0.5	0.4	—	0.4	—	1.4
	Terminal	0.6	0.6	1.0	2.2	6.3	2.1
	4-Linked	13.6	14.7	17.0	32.0	15.0	6.9
Mannosyl	3,4-	38.0	39.8	20.3	17.8	9.5	6.9
	Terminal	—	—	—	—	0.6	1.0
	4-Linked	0.6	—	0.5	0.5	6.5	20.8
Galactosyl	6-	—	—	—	—	0.6	0.4
	4,6-	—	—	—	—	0.4	0.6
	Terminal	2.9	3.0	3.3	3.9	0.5	6.7
Glucosyl	2-Linked	—	—	—	—	0.6	0.4
	4-	6.1	0.4	1.1	1.8	1.8	1.5
	6-	—	—	0.3	—	1.1	1.6
	2,4-	—	—	—	—	0.7	1.0
	3,4-	—	—	—	0.1	—	1.1
	3,6-	0.2	0.5	—	0.2	—	0.2
	4,6-	—	—	—	0.1	—	0.6
Glucosyl	Terminal	0.6	0.6	1.6	0.5	0.3	0.4
	3-	0.8	1.1	3.4	1.8	0.8	0.6
	4-	5.0	4.8	26.7	13.5	26.8	25.1
	4,6-	—	—	3.2	12.4	17.0	7.9
	2,4,6-	—	—	—	0.3	—	—
	3,4,6-	—	—	—	—	2.0	—

*Extractions with CDTA omitted in 1994.

were also detected in 1 M KOH and 4 M KOH extracts, indicating the presence of xyloglucan. 4-Linked mannosyl residues in 4 M KOH extracts is due to the presence of glucomannan. Such linkages probably indicate the presence of an arabinoxylan and of the above hetero-linked glucan. There was a difference in the glucan content of cell-walls prepared in 1993 and in 1994. The content of (1 → 3, 1 → 4)-β-D-glucan was reported to decrease during elongation of *Avena* coleoptiles. Because bamboo shoots elongate quite rapidly, the decrease rate of the glucan is supposed to be remarkable. Therefore, the time of harvest probably affected the content. The glycosyl composition of the 1 M KOH-I extract prepared in 1993 was composed mainly of 3-linked (19.1%) and 4-linked (52.2%) glucosyl residues. Purification of (1 → 3, 1 → 4)-β-D-glucan was performed from the extract. As arabinoxylan contains 4-O-methylglucuronic acid residues, the greater part of arabinoxylan contaminant can be removed by anion-exchange chromatography. The 1 M KOH-I extract was separated on a QAE sepharose A-25 column (Fig. 1). Fr. I consisted mainly of (1 → 3, 1 → 4)-β-D-glucan and Fr. II was arabinoxylan. Fr. I was further fractionated on a Bio-Gel A-5m column. However, a small amount of xylose of arabinoxylan still remained. To remove arabinoxylan completely, the fractions eluted from a Bio-Gel A-5m column were digested with purified xylanase.

Arabinoxylan was destroyed by the enzymic treatment and the polysaccharide consisting only of 3- and 4-linked glucosyl residues was obtained. The results obtained indicate that (1 → 3, 1 → 4)-β-D-glucan exists in bamboo shoot cell-walls independently of arabinoxylan, xyloglucan and glucomannan. The M_r distribution of the (1 → 3, 1 → 4)-β-D-glucan was determined on a Bio-Gel A-5m column (Fig. 2). The (1 → 3, 1 → 4)-β-D-glucan was eluted between the pullulan standard P-400 (M_r 3800 000) and P-100 (M_r 100 000). The glucose contents of Fr. A and B were more than 99.5%. The specific rotation of the glucan was -12.4. The fractions were composed of 21.0–28.6% of 3-linked glucosyl and 71.4–79.0% of 4-linked glucosyl residues (Table 4), indicating that about 1/2–1/4 of the glycosyl linkages were →Glc-(1 → 3)-Glc→ and that other glycosyl linkages were →Glc-(1 → 4)-Glc→. The M_r range of the glucan from bamboo shoot cell-walls is thus similar to the (1 → 3, 1 → 4)-β-D-glucan from other monocot cell-walls [14]. The ¹³C-NMR spectrum of the glucan showed the presence of the signals at δ 85.9 and 80.5 (data not shown), which were assigned to C-3 and C-4 of -β-(1 → 3)-linked and -β-(1 → 4)-linked glucosyl residues, respectively [15]. The obtained glucan was concluded to be (1 → 3, 1 → 4)-β-D-glucan. The (1 → 3, 1 → 4)-β-D-glucan (Fraction A in Fig. 2) was degraded with purified cellulase {endo-β-

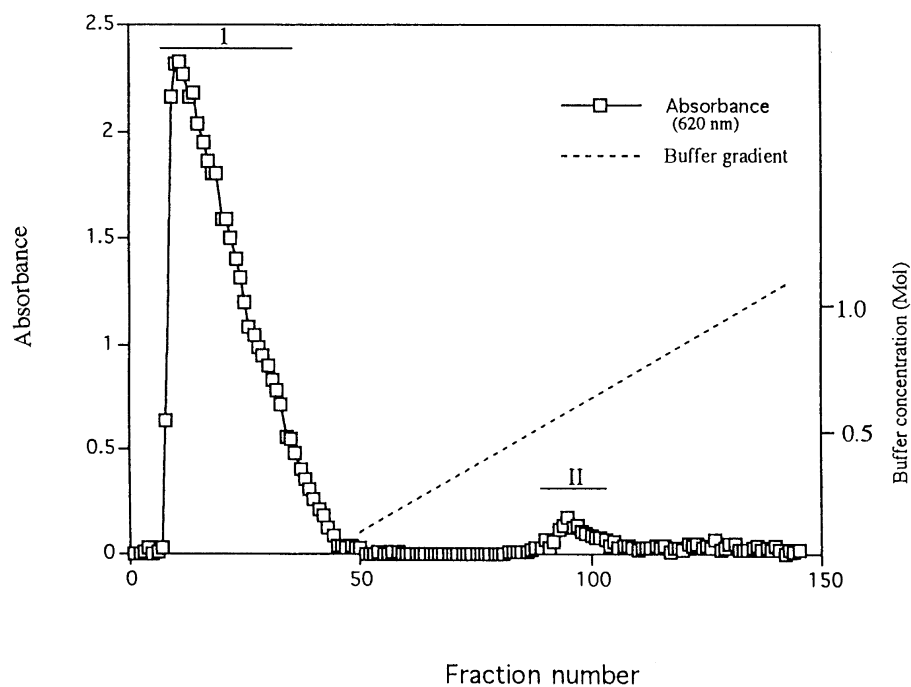


Fig. 1. QAE-Sepadex A-25 anion-exchange chromatography of 1 M KOH-I extract (1993) of bamboo shoot cell-walls. Fractions (1.5 ml) were collected and portions (20 μ l) assayed colorimetrically (OD_{620}) for hexoses by the anthrone method. Fractions I and II (shown by the bars) were pooled.

(1 \rightarrow 4)-glucanase} and the resulting (1 \rightarrow 3, 1 \rightarrow 4)- β -D-glucan oligosaccharides were structurally characterized. The enzymatic hydrolyzates were fractionated on a Bio-Gel P-2 column to give three fractions (A-I, A-II and A-III, Fig. 3). Fr. A-III was glucose. Frs A-I and A-II were reduced with NaBD₄ and per-*O*-methylated. Then, the gly-

cosyl sequences of the resulting per-*O*-methylated mono- and oligoglucoosyl alditols were determined by GC-MS. Fraction A-II was identical to cellobiose by comparison of mass spectral fragmentation patterns of the derivative with the authentic compound. These results indicate the presence of \rightarrow 4)-Glc-(1 \rightarrow 4)-Glc-(1 \rightarrow . FAB-MS of the per-*O*-

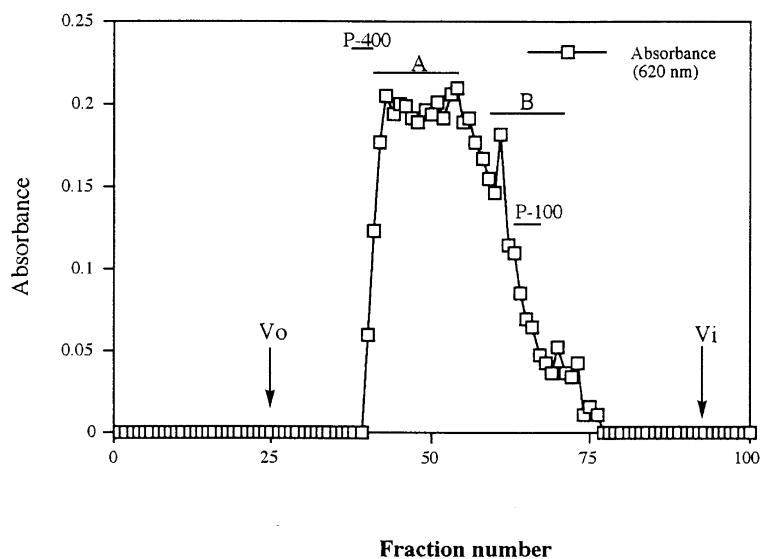


Fig. 2. Bio-Gel A-5m gel-permeation chromatography of products generated by xylanase treatment of Fraction 1 (Fig. 1). Fractions (1.5 ml) were collected and portions (20 μ l) assayed colorimetrically (OD_{620}) for hexoses by the anthrone method. Fractions A and B (shown by bars) were pooled. P-400 and P-100 showed the positions of the pullulan standards (Shodex). The M_r s of P-400 and P-100 were 380 000 and 10 000, respectively.

Table 4. Glycosyl linkage composition of (1 → 3, 1 → 4)-β-D-glucan purified from 1 M KOH-I fraction of bamboo shoot cell-walls prepared in 1993

Glycosyl Residue	Linkage	Fraction A* mol %	Fraction B* mol %
Glucosyl	3-linked	28.6	21.0
	4-linked	71.4	79.0

*see Fig. 2.

methylated oligoglycosyl alditol from Fr. A-I gave an intense peak at m/z 527 $[M + Na]^+$, indicating that it consisted of three hexosyl residues. GC-EI-mass spectrometry of the per-*O*-methylated diglycosyl alditol of fraction I gave ions at m/z 500 (baldJ₁, 3%), m/z 440 (baldJ₂, 5%), m/z 423 (baA₁, 26%), m/z 391 (baA₂, 12%), m/z 359 (baA₃, 24%), m/z 236 (aldJ₂, 58%), m/z 282 (aldJ₀, 16%), m/z 219 (aA₁, 100%) and m/z 155 (aA₃, 22%); nomenclature of fragment ions is according to Kovacic *et al.* [16, 17]. The presence of aldJ₀ ion (m/z 282) and the absence of aldJ₁ ion (m/z 296) showed that the compound contained a (1 → 3) linkage [18] and that the structure of Fr. A-I was Glc-(1 → 4)-Glc-(1 → 3)-Glc. The presence of (1 → 4) and (1 → 3) linkages was further confirmed by methylation analysis (data not shown). These results show that fraction I (Fig. 3) was derived from the structure →4)-β-D-Glc-(1 → 4)-β-D-Glc-(1 → 3)-β-D-Glc-(1 → . The trisaccharides and disaccharide containing adjoining two 3-linked glucosyl residues were detected in Frs A-I and A-II. Glycosyl linkage analysis showed that the contents of 4-linked glucosyl residues of the glucan were from 71.4 to 79.0% (Table 4). These results suggest that the tentative structure of the (1 → 3, 1 → 4)-β-D-glucan from

bamboo shoot cell-walls is →3)-β-D-Glcp-[-(1 → 4)-β-D-Glcp-]₂₋₄-(1 → .

Kato *et al.* previously obtained similar β-D-glucan from immature barley and isolated the oligosaccharides composed of 3-linked and 4-linked glucosyl residues from bamboo shoot cell-walls [6, 9]. These glucans, however, contained a small amount of arabinosyl, xylosyl and galactosyl residues. We were successful in purifying (1 → 3, 1 → 4)-β-D-glucan from an alkaline extract (1 M KOH-I). The results show that the glucan existed in monocot cell-walls independently of arabinoxylan and xyloglucan.

The extracts with 4 M KOH-I and -II of 1994 (Table 3) contained the glycosyl linkages of xyloglucan, namely, 4- and 4,6-linked glucosyl residues. Xyloglucan was purified from the 4 M KOH-I extract of 1994. Most of the arabinoxylan contaminants were separated on a QAE Sephadex A-25 anion-exchange column. The main glycosyl linkages of the obtained fractions were terminal arabinosyl, 4- and 3,4-linked xylosyl, 4- and 4,6-linked glucosyl residues, showing that arabinoxylan still remained. Attempts to fractionate xyloglucan by forming an iodide-complex [19] were unsuccessful. The fraction was further separated on a Bio-Gel A-5m column to give three fractions (Fig. 4). All of these fractions contained both glycosyl linkages of arabinoxylan, namely, 4-linked xylosyl residues, and those of xyloglucan, indicating that anion-exchange chromatography, selective precipitation with iodide and gel-permeation chromatography was not effective in the purification of xyloglucan from bamboo shoot cell-walls.

Fraction III (Fig. 4) was digested thoroughly with purified xylanase and the enzymatic degradation products sub-fractionated, giving two frac-

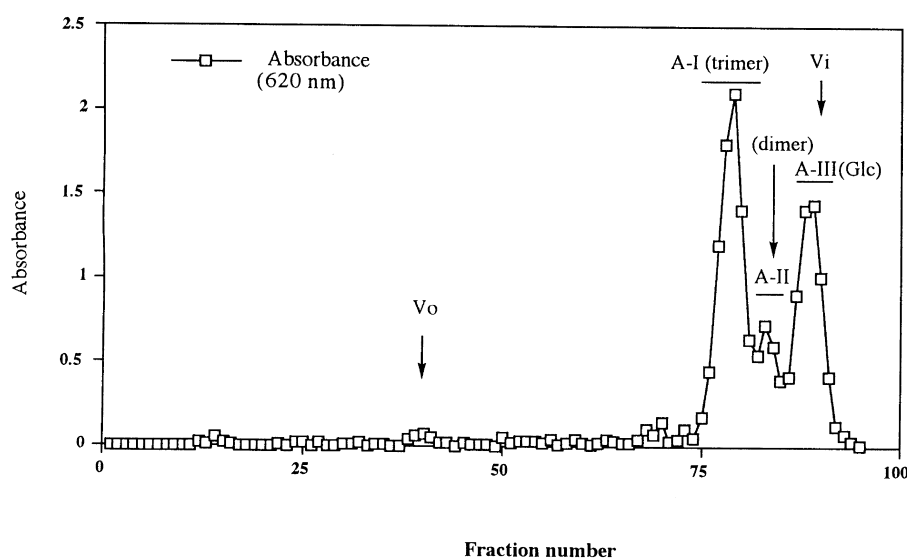


Fig. 3. Bio-Gel P-2 gel-permeation chromatography of products generated by cellulase treatment of the (1 → 3, 1 → 4)-β-D-glucan. Each fraction (1.5 ml) was assayed colorimetrically (OD_{620}) for hexoses by the anthrone method. Fractions A-I, -II and -III (shown by bars) were pooled.

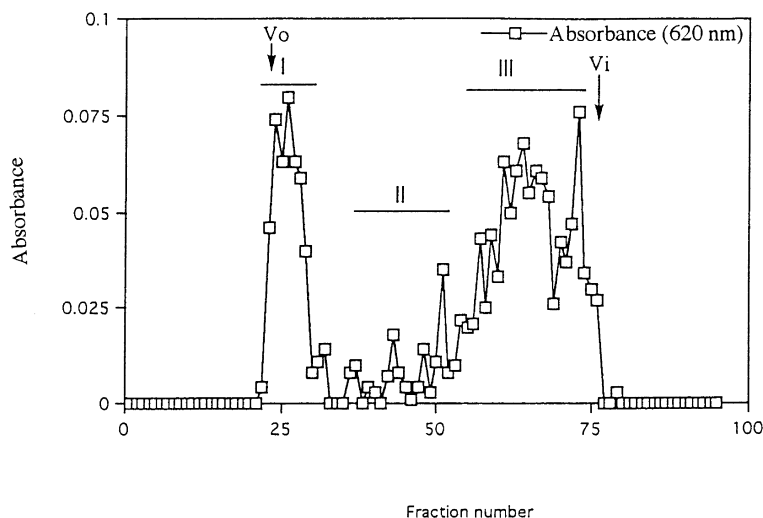


Fig. 4. Bio-Gel A-5m gel-permeation chromatography of fraction obtained from 4 M KOH-I extract (1994) of bamboo shoot cell-walls by QAE-Sephadex A-25 anion-exchange chromatography and selective precipitation with iodide. Each fraction (1.5 ml) was assayed colorimetrically (OD_{620}) for hexoses by the anthrone method. Fractions I, II and III (shown by bars) were pooled.

tions, namely, Fraction III-A (polysaccharides, void volume fraction) and III-B (oligosaccharides, low M_r fraction) on a Bio-Gel P-2 column. Fraction III-B contained oligosaccharides from arabinoxylan (Table 4), showing that the arabinoxylan contaminants were mainly degraded. On the other hand, Fraction III-A still contained glycosyl linkages of both xyloglucan and arabinoxylan, suggesting that residual arabinoxylan could not be degraded completely, even by xylanase treatment. Fraction III-A was hydrolyzed with cellulase and the M_r s of the obtained oligosaccharides were determined by FAB-mass spectrometry. M_r s $\{(M + Na)^+\}$ equivalent to dicot xyloglucan oligosaccharides, such as 1085, 1247, 1393, 1409 and 1555, were observed; these correspond to XXXG, XXLG, XXFG, XLLG and XLFG, respectively [20]. Furthermore, M_r s $\{(M + Na)^+\}$ different from those of ordinary dicot xyloglucan oligosaccharides, namely, 1115, 1217 and 1277, also appeared. They correspond to M_r s of (pent)₂(hex)₅, (pent)₄(hex)₄ and (pent)₂(hex)₆, respectively.

Xyloglucan prepared from bamboo shoot cell-walls by anion-exchange chromatography, precipitation with iodide and gel-permeation chromatography contained arabinoxylan. The arabinoxylan was not removed completely by hydrolysis with xylanase. Digestion of the xyloglucan with cellulase produced oligosaccharides whose glycosyl compositions were different from ordinary xyloglucan oligosaccharides. Thus, the structure of xyloglucan from bamboo shoot cell-walls is slightly different from that of sycamore xyloglucan and, furthermore, does not possess any linkage between xyloglucan and arabinoxylan.

EXPERIMENTAL

Plant material

Fresh young bamboo (*Phyllostachys edulis* A. and C. Riv.) shoots were purchased in May 1993 and 1994 from a local store (Ibaraki Prefecture). Cell-walls were prepared as alcohol-insoluble residue as described previously [21] and subjected to sequential extraction.

Sequential extraction

Cell-wall polysaccharides were extracted by the method described in ref. [13]. Cell-walls were treated with 0.05 M CDTA at 20° (CDTA-I) and then at 20° (CDTA-II). The CDTA-treated cell-walls were subjected to sequential extraction with 0.05 M Na₂CO₃ containing 20 mM NaBH₄ under N₂ at 1° (Na₂CO₃-I) and with 0.05 M Na₂CO₃ under N₂ at 20° (Na₂CO₃-II). The extracted residue was sequentially treated with 100 ml of 1 M KOH containing 10 mM NaBH₄ under N₂ at 1° (1 M KOH-I), with 1 M KOH under Ar at 20° (1 M KOH-II), with 4 M KOH containing 10 mM NaBH₄ under Ar at 20° (4 M KOH-I), and with 4 M KOH containing 3–4% boric acid under Ar at 20° (4 M KOH-II). Extraction with CDTA was omitted for bamboo shoots purchased in 1994, because it was difficult to remove CDTA completely from extracts by dialysis.

Ion-exchange chromatography

A fr. dissolved in 10 mM imidazole-HCl buffer (pH 7) was loaded onto a QAE Sephadex A-25 column (1.5 cm i. d. × 80 cm, Pharmacia) equilibrated with the same buffer. The column was washed with 250 ml of the same buffer and then eluted with linear gradient from 10 mM to 1.5 M. Eluted frs

(1.5 ml) were collected and assayed by the anthrone method [23]. Anthrone-positive frs were pooled, dialyzed against de-ionized H₂O and lyophilized.

Purification of xyloglucan by precipitation as iodide complex

Purification was carried out by using the procedure of Ref. [22]. The fr. containing xyloglucan was dissolved in CaCl₂ soln (SG 1.3), and insol. materials removed by centrifugation. Excess of iodide-potassium iodide soln (3% I₂ dissolved in 4% soln of KI) was added to the soln and kept at 4° for 2 h. Prpites were collected by centrifugation and dissolved in hot H₂O. The soln was then titrated with Na₂S₂O₃ soln until the brown colour disappeared. The obtained soln was dialyzed exhaustively against de-ionized H₂O, concd to small vol. under red. pres. and lyophilized.

Gel-permeation chromatography

A fr. dissolved in 50 mM acetate buffer (pH 5) was loaded onto a Bio Gel A-5m or P-30 or P-2 column (1.5 cm i. d. × 80 cm, Bio-Rad). Eluted frs (1.5 ml) were collected and assayed by the anthrone method. Anthrone-positive frs were pooled, passed through a column of Dowex 50W (H⁺) and lyophilized.

Enzymatic hydrolysis

Polysaccharides were dissolved in 50 mM NaOAc buffer (pH 5) and purified enzyme [cellulase from *Trichoderma viride* {endo-β-(1 → 4)-glucanase, (Megazyme)} or xylanase from *Trichoderma viride* (Megazyme)] was added to the sample soln (5 units of enzyme g⁻¹ of sample). After adding a few drops of toluene, enzymatic hydrolysis was carried out at 30°. After 48 hr, the sample soln was heated at 100° for 5 min, cooled, passed through a column of Dowex 50W-X8 cation-exchange resin (Bio-Rad) to remove Na⁺ and lyophilized.

Colorimetric assay

Hexose contents of column frs were determined by the anthrone method.

Glycosyl composition

Neutral and acidic glycosyl compositions were determined as TMSi ethers of Me glycosides after methanolysis with 5% HCl-MeOH soln at 80° for 16 hr [24]. Derivatives obtained were analyzed as described in Ref. [25].

Methylation analysis

Glycosyl linkage composition was determined by GC and GC-MS of partially *O*-methylated alditol acetates. Per-*O*-methylation was performed by a modification [26] of the Hakomori procedure [27]. Per-*O*-methylated polysaccharides were purified using Sep-Pak C₁₈ cartridges [28]. Per-*O*-methylated

polysaccharides were hydrolyzed with 2 M TFA at 121° for 1 hr and converted into the corresponding alditol acetate derivatives. Analyses were performed as described in Ref. [25].

Preparation of per-O-methylated oligoglycosyl alditols

Oligosaccharides produced from (1 → 3,1 → 4)-β-D-glucan by cellulase treatment were reduced with NaBD₄. The resulting sample was desalted on a Dowex 50W-X8 (H⁺) column, MeOH added and the sample evapd to dryness. The oligoglycosyl alditols obtained were per-*O*-methylated using a modified Hakomori procedure. The *M_r* values of the resulting per-*O*-methylated oligoglycosyl alditols were determined by GC-MS and FAB-MS.

Mass spectrometry

Per-*O*-methylated oligoglycosyl alditols were analyzed by GC-MS using a DB-1 capillary column (30 m × 0.25 mm i.d.) in the splitless mode with a temp. program starting at 50° for 2 min, followed by a rate of 30° min⁻¹ to 190° and, then at a rate of 6° min⁻¹ to 340°. MS were recorded at 70 eV with a source temp. of 180°. FAB-MS were recorded with an emission current of 10 mA and an acceleration volt of 3 kV. Samples were mixed with glycerol and loaded onto a stainless steel target. Ar was used as bombardment gas.

¹³C-NMR

Spectra (100 Mhz) were recorded at 27°. Samples were dissolved in D₂O (99.96 atm% D). Chemical shifts are expressed in δ with reference to external d₄-MeOH (δ 49.8).

REFERENCES

1. Fry, S. C., *Physiologia Plantarum*, 1989, **75**, 532.
2. Darvill, A. G. and Albersheim, P., *Annual Review of Plant Physiology*, 1984, **35**, 243.
3. Albersheim, P. and Darvill, A. G., *Scientific American*, 1985, **253**, 58.
4. York, W. S., Darvill, A. G. and Albersheim, P., *Plant Physiology*, 1984, **75**, 295.
5. Dey, P. M. and Brinson, K., *Advances in Carbohydrate Chemistry and Biochemistry*, 1984, **42**, 265.
6. Kato, Y., Iki, K. and Matsuda, K., *Agricultural and Biological Chemistry*, 1981, **45**, 2737.
7. Kato, Y., Iki, K. and Matsuda, K., *Agricultural and Biological Chemistry*, 1981, **45**, 2745.
8. Kato, Y., Ito, S., Iki, K. and Matsuda, K., *Plant and Cell Physiology*, 1982, **23**, 351.
9. Kato, Y., Shiozawa, R., Takeda, S., Ito, S. and Matsuda, K., *Carbohydrate Research*, 1982, **109**, 233.
10. Kato, Y. and Nevins, D. J., *Plant Physiology*, 1984, **75**, 740.

11. Loescher, W. and Nevins, D. J., *Plant Physiology*, 1972, **50**, 556.
12. Nevins, D. J., Huber, D. J., Yamamoto, R. and Loescher, W. H., *Plant Physiology*, 1977, **60**, 617.
13. Selvendran, R. R. and O'Neill, M. A., *Methods of Biochemical Analysis*, 1987, **32**, 25.
14. Henriksson, K., Teleman, A., Reinikainen, T., Jaskari, J., Teleman, O. and Poutanen, K., *Carbohydrate Polymers*, 1955, **26**, 109.
15. Bock, K., Christian, R. and Pedersen, H., *Advance in Carbohydrate Chemistry and Biochemistry*, 1985, **42**, 193.
16. Kováčik, V., Bauer, Š., Rosík, J. and Kopáč, P., *Carbohydrate Research*, 1968, **8**, 282.
17. Kováčik, V., Bauer, Š. and Rosík, J., *Carbohydrate Research*, 1968, **8**, 291.
18. Sharp, J. K. and Albersheim, P., *Carbohydrate Research*, 1984, **128**, 193.
19. Kato, Y. and Matsuda, K., *Plant and Cell Physiology*, 1976, **17**, 1185.
20. Fry, S. C., York, W. S., Albersheim, P., Darvill, A. G., Hayashi, T., Joseleau, J.-P., Kato, Y., Lorence, E. P., Maclachlan, G. A., McNeil, M., Mort, A. J., Reid, J. S. G., Seitz, H. U. S., Selvendran, R. R., Voragen, A. G. J. and White, A. R., *Physiologia Plantarum*, 1993, **89**, 1.
21. Ishii, T. and Hiroi, T., *Carbohydrate Research*, 1990, **196**, 175.
22. Gaillard, B. D. E., *Nature*, 1961, **191**, 1295.
23. Dishe, Z., *Methods in Carbohydrate Chemistry*, 1962, **1**, 477.
24. Sweeley, C. C., Bentley, R., Makita, M. and Wells, W. W., *Journal of American Chemical Society*, 1963, **85**, 2497.
25. York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T. and Albersheim, P., *Methods in Enzymology*, 1986, **118**, 3.
26. Sandford, P. A. and Conrad, H. E., *Biochemistry*, 1966, **5**, 1508.
27. Hakomori, S., *Journal of Biochemistry*, 1964, **55**, 205.
28. Waeghe, T. J., Darvill, A. G., McNeil, P. and Albersheim, P., *Carbohydrate Research*, 1983, **123**, 281.